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1 Please give the title of the invention **PHARMACEUTICALS**

❷ Applicant's details

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2b If you are applying as an individual or one of a partnership please give in full:

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Forenames

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ADP number *(if known)* 05791553001

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Agent's address: **CORPORATE INTELL PROPERTY**
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SB HOUSE
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Postcode **TW8 9BD**
Agent's ADP number 065567400021

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4. Agent's or applicant's reference number *(if applicable)*

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15(4) (Divisional) ☐ 8(3) ☐ 12(6) ☐ 37(4) ☐

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❼

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Inventorship

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Continuation sheets for this Patents Form 1/77 ☐

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Abstract -- Drawing(s) --

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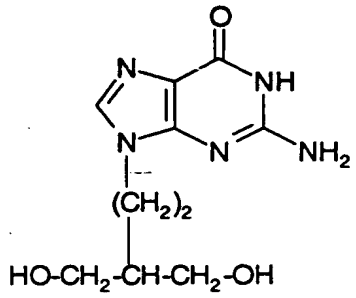
PHARMACEUTICALS

This invention relates to treatment of human immunodeficiency virus (HIV) and hepatitis B virus (HBV) infection.

When used herein, 'treatment' includes prophylaxis as appropriate.

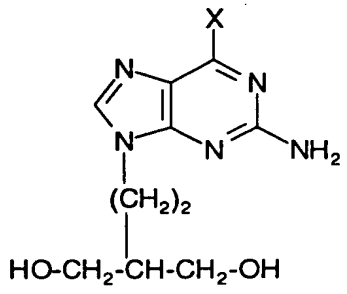
EP-A-141927 (Beecham Group p.l.c.) discloses penciclovir, the compound of formula (A):



(A)

and salts, phosphate esters and acyl derivatives thereof, as antiviral agents. The sodium salt hydrate of penciclovir is disclosed in EP-A-216459 (Beecham Group p.l.c.). Penciclovir and its antiviral activity is also disclosed in Abstract P.V11-5 p.193 of 'Abstracts of 14th Int. Congress of Microbiology', Manchester, England 7-13 September 1986 (Boyd et. al.).

Orally active bioprecursors of the compound of formula (A) are of formula (B):



(B)

and salts and derivatives thereof as defined under formula (A); wherein X is C_{1-6} alkoxy, NH_2 or hydrogen. The compounds of formula (B) wherein X is C_{1-6} alkoxy or NH_2 are disclosed in EP-A-141927 and the compounds of formula (B) wherein X is hydrogen, disclosed in EP-A-182024 (Beecham Group p.l.c.) are preferred prodrugs. A particularly preferred example of a compound of formula (B) is that wherein X is hydrogen and wherein the two OH groups are in the form of the acetyl derivative, described in Example 2 of EP-A-182024, hereinafter referred to as famciclovir.

EP-A-388049 (Beecham Group p.l.c.), discloses the use of penciclovir/famciclovir in the treatment of hepatitis B virus infection.

The antiviral activity against hepatitis B virus appears to be dependent on intracellular formation of PCV-triphosphate (PCV). The triphosphate derivative of penciclovir inhibits the RNA-directed DNA polymerase (reverse transcriptase) activity of human immunodeficiency virus type 1 (HIV-1). The reverse transcriptase of HIV-1 is a virus-encoded enzyme essential for the conversion of genomic RNA into proviral ds-DNA.

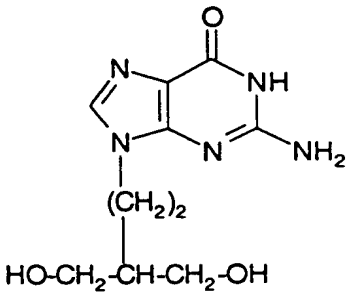
It has now been shown that the (R)-enantiomer of PCV-TP is more active than the (S)-enantiomer in respect of inhibition of HBV DNA polymerases and in respect of inhibition of HIV-1 reverse transcriptase.

Accordingly, the present invention provides a method of treatment of:

i) HIV-1 infections in mammals, including humans, which mammals are infected with herpesviruses; or

ii) HBV infections in mammals, including humans;

which method comprises the administration to the human in need of such treatment, an effective amount of the (R)-enantiomer of the triphosphate of a compound of formula (A):



(A)

or a pharmaceutically acceptable salt thereof.

The (R)-PCV-TP is administered in the form of a compound which is a bioprecusor to allow absorption and penetration through the cell wall. Selectivity for the virus infected cell, especially HIV infected cells, can be achieved by selecting a bioprecursor which is activated preferentially by the virally encoded protease.

The compound may be administered by the oral route to humans and may be compounded in the form of syrup, tablets or capsule. When in the form of a tablet, any pharmaceutical carrier suitable for formulating such solid compositions may be used, for example magnesium stearate, starch, lactose, glucose, rice, flour and chalk. The compound may also be in the form of an ingestible capsule, for example of gelatin, to contain the compound, or in the form of a syrup, a solution or a suspension. Suitable liquid pharmaceutical carriers include ethyl alcohol, glycerine, saline and water to which flavouring or colouring agents may be added to form syrups. Sustained release formulations, for example tablets containing an enteric coating, are also envisaged.

For parenteral administration, fluid unit dose forms are prepared containing the compound and a sterile vehicle. The compound depending on the vehicle and the concentration, can be either suspended or dissolved. Parenteral solutions are normally prepared by dissolving the compound in a vehicle and filter sterilising before filling into a suitable vial or ampoule and sealing. Advantageously, adjuvants such as a local anaesthetic, preservatives and buffering agents are also dissolved in the vehicle. To enhance the stability, the composition can be frozen after filling into the vial and the water removed under vacuum.

Parenteral suspensions are prepared in substantially the same manner except that the compound is suspended in the vehicle instead of being dissolved and sterilised by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the compound of the invention.

As is common practice, the compositions will usually be accompanied by written or printed directions for use in the medical treatment concerned.

A suitable dosage unit might contain from 50mg to 1g of active ingredient, for example 100 to 500mg. Such doses may be administered 1 to 4 times a day or more usually 2 or 3 times a day. The effective dose of compound will, in general, be in the range of from 0.2 to 40mg per kilogram of body weight per day or, more usually, 10 to 20 mg/kg per day.

The present invention also provides the use of the (R)-enantiomer of the triphosphate of a compound of formula (A) in the preparation of a medicament for use in the treatment of:

i) HIV-1 infections in mammals, including humans, which mammals are infected with herpesviruses; or

ii) HBV infections in mammals, including humans.

Such treatment may be carried out in the manner as hereinbefore described.

The present invention further provides a pharmaceutical composition for use in the treatment of:

i) HIV-1 infections in mammals, including humans, which mammals are infected with herpesviruses; or

ii) HBV infections in mammals, including humans;

which comprises an effective amount of the (R)-enantiomer of the triphosphate of a compound of formula (A), and a pharmaceutically acceptable carrier.

Such compositions may be prepared in the manner as hereinafter described.

The biological data describing the activity of (R)-PCV-TP is described by Shaw *et al*, Zoulim *et al* and Schinazi *et al* in 'Antiviral Research' 1995, Supplement 1. A photocopy of material to be included in this Supplement is attached to the present specification.

WHAT IS CLAIMED IS:

The subject matter of the invention described herein in all aspects and embodiments.

Preferential Inhibition of Human Hepatitis B Virus (HBV) DNA Polymerase by the (R)- Enantiomer of Penciclovir Triphosphate. SU SAN MOK, TIM SHAW* and STEPHEN LOCARNINI. Victorian Infectious Diseases Reference Laboratory, Fairfield Hospital, Fairfield, 3078, Australia.

Penciclovir (PCV), a deoxyguanosine analogue, has potent antiviral activity against herpes- and hepadnaviruses. Efficacy against chronic HBV has been demonstrated in recent clinical trials of famciclovir, the oral form of PCV. Antiviral activity appears dependent on intracellular formation of PCV-triphosphate (PCV-TP). In both control and HBV-transfected liver cells in vitro, PCV-TP concentrations of about 0.04 μM were achieved, indicating cellular phosphorylation of PCV. The (S)-enantiomer of PCV-TP is preferentially formed in herpesvirus-infected cells and is the more active against HSV and VZV. By contrast, we found (R)-PCV-TP to be the more potent inhibitor of HBV DNA polymerases in vitro. In standard HBV DNA polymerase assays, the Km for dGTP was 0.09 μM and the Kis for (R)- and (S)- Pcv-TP were 0.03 μM and 0.04 μM respectively. Corresponding IC50's for each enantiomer in the presence of 0.01 μM dGTP were 2.5 μM and 11 μM, compared to 10 μM for acyclovir-TP. These data suggest differences between herpes- and hepadnaviral polymerases and provide a mechanistic basis for the potent activity of PCV against HBV polymerase.

35th ICAAC, San Francisco, Calif rnia

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Inhibitory Effect of Penciclovir on the Primi.. Hepadnavirus Reverse Transcription.

F. ZOULIM*, E. DANNAOUI, C. TREPO. INSERM U271, Lyon, France.

Famciclovir, the oral form of penciclovir (PCV), has been shown to have an inhibitory effect on hepatitis B virus (HBV) replication in chronically infected humans and in animal models. We used an in vitro translation reaction for the expression of an enzymatically active Duck HBV reverse transcriptase to characterize the mechanism of action of PCV. Using this system, we have already demonstrated that DHBV DNA synthesis is initiated by the formation of a covalent bond between the polymerase and dGMP, followed by the addition of T-A-A in a template dependent manner (J.Virol. 68 : 6-13). Several acyclic guanosine analogs were tested for their efficacy to inhibit the priming of DHBV reverse transcription in an in vitro assay. We found that acyclovir-TP (ACV), R-PCV-TP, S-PCV-TP but also ddG-TP and 2'-carboxydcoxyguanosine (2'-CDG) could inhibit reproducibely minus strand DNA synthesis at different extent. Interestingly, R-PCV-TP was more efficient than S-PCV-TP in inhibiting DHBV reverse transcription. The inhibitory effect of these compounds against the incorporation of the first nucleotide of minus strand DNA, dGMP, was similar to that observed with minus strand DNA elongation. Both ACV-TP and R-PCV-TP inhibited dramatically the incorporation of dATP whereas 2'-CDG which was the most efficient competitor of dGMP incorporation did not.
We demonstrated that PCV-TP inhibits hepadnavirus reverse transcription in a chirally dependent manner, by inhibiting the synthesis of the short DNA primer. Our data obtained with the inhibition of the enzymatic activity of the DHBV polymerase provide a new insight on the mechanism of action of PCV on HBV replication.

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Abstract Title

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Taken from draft manuscript by Zoulim.

III. RESULTS

1) An in vitr assay for the expression of an enzymatically active DHBV e transcriptase

We had previously shown that the DHBV polymerase expressed in vitro in a reticulocyte lysate is enzymatically active if the template for the initiation of RT, ie ε, is provided during the translation of the viral enzyme. In figure 2, we show that, when the RNA sequence A which contains ε, DR1 and the 5' flanking region, is coexpressed in trans, the efficiency of reverse transcription was enhanced by approximately 50%. A similar increase of enzymatic activity was observed on both the priming reaction and DNA chain elongation.

2) Inhibitory effect of pyrophosphate analogs on the DHBV reverse transcriptase

Pyrophosphate analogs have been shown to be potent inhibitors of DNA polymerases as well as of reverse transcriptases (1). We have studied the inhibitory effect of PAA (phosphono acetic acid) and that of PFA (phosphonoformic acid) on the DHBV reverse transcription. As shown in figure 3, PAA did not inhibit minus strand DNA synthesis even at high concentration (1mM). By contrast, PFA showed a very potent inhibitory effect on DNA chain elongation but not on the priming reaction, at a concentration of 1 mM. This PFA concentration has been previously used to block DHBV reverse transcription in tissue culture cells (4).

3) Inhibitory effect of dGTP analogs on the elongation of minus strand DNA (reverse transcription)

We have compared the inhibitory activity of dGTP analogs on the elongation of minus strand DNA (see figure 4). Extended DNA chain is covalently linked to the viral polymerase. This allows for its study through 0.1% SDS-10% polyacrylamide gels. Viral DNA synthesis was

analyzed by the incorporation of dNTPs (dATP, TTP, dCTP) and radiolabelled α-^{32}P-dGTP. The level of α-^{32}P-dGTP incorporation was also measured by a dot assay on DE-81 filters. As shown in figure 4, the incorporation of α-^{32}P-dGTP in the presence of increasing concentrations of ACV-TP, R-PCV-TP, S-PCV-TP, ddG-TP and CDG-TP was reproducibly inhibited. At a concentration of 100 µM, ddG-TP, ACV-TP and R-PCV-TP showed an almost complete inhibition effect (> 75 %). S-PCV-TP was the less active compound and very high concentrations (1 mM) were needed to achieve a significant inhibition of reverse transcription (> 75 %). CDG was the most active compound since the same order of inhibition could be obtained at a concentration of 10 µM. The IC50 of ACV-TP, R-PCV-TP, ddG and CDG-TP was approximately 7 uM, 8 uM, 20 uM and < 1 uM , respectively. CDG was the most efficient compound followed by ACV and PCV. **R-PCV was more efficient than S-PCV.**

4) Effect of dGTP analogs on the incorporation of the first nucleotide of minus strand DNA, dGTP

In this experiment, we tested the inhibitory effect of this different analogs on DNA-priming (ie, incorporation of the first nucleotide of minus strand DNA, dGTP). The DHBV polymerase was incubated only with 32P-dGTP (0.15 µM final concentration, 3000 Ci/mmole). ACV, R-PCV, S-PCV, ddG and CDG were tested in the same range of concentrations. Results were in agreement with that obtained with viral DNA chain extension (figure 5).

ACV and R-PCV did inhibit the incorporation of the first nucleotide, dGTP. The IC50 of these two compounds was approximately 20 uM which was higher than that obtained with the DNA chain elongation. **Again R-PCV was more effective on the priming reaction than S-PCV which had an IC50 higher than 1 mM.** ddG was more efficient on the priming reaction than on DNA chain elongation since the IC50 for the priming reaction was approximately 1 uM. CDG was the most potent inhibitor of dGTP incorporation, since the inhibition was almost complete at a concentration of 10 µM.

ICAR Poster by R. Schinazi.

Inhibition of Viral Enzymes by PCVTP

Enzymic studies showed that racemic PCVTP was an efficient inhibitor of HIV-1 reverse transcriptase (RT) and that (*R*)-PCVTP was at least 20-fold more potent than the corresponding (*S*)-enantiomer using an RNA-dependent template with HIV RT (Figure 2 and Table 1). Using a DNA template, the enantiomers were essentially inactive when tested up to 10 μM. In contrast, using an M13mp18(+) strand DNA template, chain termination was observed at 10 μM. Both the (*R*)- and (*S*)-PCVTP inhibited DNA elongation, but it appears that the (*R*)-enantiomer is less selective (in addition to stops at G bases, stops at other bases were observed). Of interest was the finding of more G stops in a 130 base stretch with either PCVTP enantiomers and ddGTP than with ACVTP. As anticipated, chain termination only occurred at G bases with both ACVTP and ddGTP.

Table 1. Summary of Inhibition of Viral Enzymes by Penciclovir Triphosphate

Compound	Recombinant HSV-1[a] Activated DNA $IC_{50} \pm$ S.D., µM	HSV-2/Cell Derived[b] Activated DNA $IC_{50} \pm$ S.D., µM	Recombinant p66/51 HIV-1 RT rCdG[c] $IC_{50} \pm$ S.D., µM	Recombinant p66/51 HIV-1 RT dCdG[d] $IC_{50} \pm$ S.D., µM
Racemic PCVTP	15.5	26.7	2.7 ± 1.6	> 10
(*R*)-PCVTP			0.91 ± 0.76	> 10
(*S*)-PCVTP			8.3 ± 4.5	> 10
(*S*)-PCVTP HSV-infected cell derived	54.2	23.6	> 10	ND
ACVTP	0.14 ± 0.11	0.051	0.060 ± 0.026	1.7
ddGTP	51.5 ± 31.9	ND	0.025 ± 0.037	0.021
PFA	8.9	ND	9.4 ± 3.2	ND

[a] 75 µl reaction mixture (1 unit enzyme, 50 mM Tris, pH 8.0, 150 mM KCl, 5 mM $MgCl_2$, 0.5 mM DTT, 0.5 mg/ml BSA, 0.1 mM each dATP, dCTP, dTTP, 0.2 mg/ml activated DNA, 1 µM ^{3}H-dGTP, 8 Ci/mmol) incubated 60 min. at 37°C, stopped with 5% TCA/0.05% sodium pyrophosphate, harvested onto glass fiber sheets using Packard Filtermate 196 harvester and counted using Packard Matrix 9600 direct beta counter.

[b] Methodology as in *a*, except ^{3}H-dGTP activity: 42 Ci/mmole.

[c] Methodology as in a, except 100 µl reaction mixture: 1 unit enzyme, 100 µM Tris pH 8.0, 50 mM KCl, 2 mM $MgCl_2$, 5 mM DTT, 0.05 U/ml $(rA)_n \cdot (dT)_{12-18}$, 1 µM ^{3}H-dGTP, 8 Ci/mmole

[d] Methodology as in *c*, except template-primer: 0.05 U/ml $(dC)_n \cdot (dG)_{12-18}$